UNIVERSITY OF PENNSYLVANIA

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CHARLES RITTENHOUSE PENDLETON

A THESIS

IN PSYCHOLOGY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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THE COLD RECEPTOR

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The original object of this study was to apply histological tests to the Von Frey theory of receptors for the four modality sensitivity of the skin. The theory was propounded a number of years ago[1] and yet no direct histological demonstrations have been made. In fact it appears that there are no adequate studies of the nerve endings of the hairy parts of the skin. Ruffini[2] says that all histological studies of the skin of man and the higher vertebrates worth mentioning have been confined to the palms of the hands and fingers, the soles of the feet, the eye, the generative organs, the lips and similar hairless parts. Very little seems to have been done since Ruffini's time.[3]

Further, the theory seems to have an illogical coupling of one kind of receptor for the conjunctiva of the eye and another kind for the other parts of the skin. It calls for the Krause end-bulbs in the conjunctiva, and the Dogiel corpuscles in the skin, which latter are assumed to be the same as the Golgi-Màzzoni corpuscles of Ruffini.[4] Two different forms for the same function is not unknown; but here the Krause end-bulb of the eye, the supposed cold organ seems to be homologous to the Meissner corpuscle of the palms, the supposed tactile organ. Krause end-bulbs lie just below the conjunctiva in the papillae, while Meissner's corpuscles occupy a corresponding position in the hairless parts of the body, *i.e.* just below the epidermis,[5] which is

*Accepted for publication May 9, 1927.

[1] M. von Frey, Beiträge zur Sinnesphysiologie der Haut, *Ber. d. königl. säch. Gesell. d. Wiss., math.-phys. Kl.*, 47, 1895, 180.

[2] A. Ruffini, Les expansions nerveuses de la peau chez l'homme et quelques autres mammifères, *Revue General d' Histologie*, 1905, 425.

[3] Luigi Luciani, *Human Physiology*, IV, tr., 1917, 55.

[4] The Golgi-Màzzoni corpuscles of Ruffini would seem to be the forms indicated. The Dogiel corpuscles according to Ruffini's nomenclature, have been reported but once, by Dogiel, and are intermediate in form between the Meissner corpuscle and the free papillary nerve ending (A. Ruffini, *op. cit.*, 435-442).

[5] L. F. Barker, *The Nervous System*, 1899, 387, fig. 243. A. S. Dogiel, Die Nervenendkörperchen (Endkolben, W. Krause), in der Cornea und Conjunctiva Bulbi des Menschen, *Arch. f. mikr. Anat.*, 37, 1891, 605. A. S. Dogiel, Die Nervenendigungen im Lidrande und in der conjunctiva palpebr. des Menschen, *Arch. f. mikr. Anat.*, 44, 1894-5, 15-25, table III, fig. 1. G. A. Piersol, *Human Anatomy*, 1907, 1016.

homologous to the conjunctiva. Further, cats and dogs have Krause end-bulbs in the dermic papillae of the soles of the feet where men and apes have Meissner corpuscles.[6]

Opposed observations. A number of years ago Donaldson,[7] using gold chloride, failed to find any organs under the temperature spots. Häggqvist,[8] using hematoxylin and eosin, found smooth muscle bundles under the cold spots. Stetson,[9] working on the hair follicle, failed to find the tactile organ. Goldscheider,[10] working with chemicals, concludes that the end-organs of temperature are not stratified and lie at the boundary between the epidermis and the corium. Dallenbach,[11] using methylene blue, failed to find any bulbs under the temperature spots.

Confirmatory observations. It may be said that all these negative findings are meaningless, especially as histology is involved in all but one of them, while on the other hand Von Frey's co-workers and students have offered several confirmatory observations that cannot be ignored.

Strughold[12] found that Krause end-bulbs were present in all parts of the mouth sensitive to cold—but the converse was not established. Strughold and Karbe[13] found that the topography of the cold sense of the conjunctiva agreed with that of the Krause end-bulbs as reported in the literature. Strughold[14] found that the threshold for the cold spots was higher in regions where the conjunctiva was thicker. Strughold and Karbe[15] colored the nerves of the conjunctiva with methylene blue and found that Krause end-bulbs thus displayed were under cold spots, the positions of which were later determined.

Plan of the present study. Having in mind these contradictory findings, the writer tried to find some solution of the problem. This work was divided into five parts. I. The comparison of the patterns of the temperature spots with those of the nerve end-organs. II. The search for Krause end-bulbs and Ruffini cylinders in the hairy parts of the skin. III. The application of Bielchowsky's method of staining to the skin. IV. The determination of the most probable depth of the cold receptors. V. An histological examination of the skin. The first three problems gave negative results. The last two, however, gave results that we believe are significant. The work is

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[6]A. Ruffini, *op. cit.*, 458-476.

[7]H. H. Donaldson, Research on the temperature-sense, *Mind*, O. S., 10, 1885, 408. f

[8]Gösta Haggqvist, Histophysiologische Studien über die Temperatursinne der Haut des Menschen, *Anat. Anz.*, 45, 1913, 46-63.

[9]R. H. Stetson, The hair follicle and the sense of pressure, *Psychol. Monog.*, 32, 1923, no. 145, 1-17.

[10]A. Goldscheider and H. Hahn, Untersuchungen über den Temperatursinn: II. Chemische Reizung und Lahmung der Temperaturnerven. Schichtungstheorie. Paradoxe Wärmeempfindung. Hitzeempfindung. *Arch. f. d. ges. Physiol.*, 206, 1924, 308-324.

[11]K. M. Dallenbach, The temperature spots and endorgans, this JOURNAL, 39, 1927, 424 ff.

[12]Hubert Strughold, Die Topographie des Kältesinnes in der Mundhöhle, *Zsch. f. Biologie*, 83, 1925, 515-534.

[13]Hubert Strughold and M. Karbe, Die Topographie des Kältesinnes auf Cornea und Conjunctiva, *Zsch. f. Biologie*, 83, 1925, 189-200.

[14]Hubert Strughold, Die Schwellen des Kältesinnes am Auge, *Zsch. f. Biologie*, 83, 1925, 201-206.

[15]Hubert Strughold and M. Karbe, Vitale Färbung des Auges und experimentelle Untersuchung des gefärbten Nervenelemente, *Zsch. f. Biologie*, 83, 1925, 297-308.

reported as a whole, for the negative results, as well as the positive, may be of some assistance to other workers in this field.

I. Comparison of the Patterns of the Temperature Spots and of the Nerve End-Organs

In the first part of the work, emphasis was laid upon technique so as to avoid two possible sources of error observed in the earlier investigations. The first of these was the possible shifting of the organs in the excision and later manipulations. Although preliminary experiments seemed to show that the cold spots did not shift even when the skin was pulled to one side, or lifted, it seemed advisable to take out large pieces of skin with several spots to see if any pattern relation between the spots and the organs could be observed. The second was the staining. It is very well known that the special nerve-stains are capricious, staining only a part of the nerve elements, and often stopping short at some point, for no discoverable reason. We, therefore, decided to use ordinary histological stains and to depend on the organized tissues constituting the nerve end-bulbs for a means of identification. Nerve end-bulbs are often seen in good preparations and are large enough to be readily detected—the smallest being several times as large as the red blood corpuscle.[16] The only tissue with which they might be confused is a section of a nerve trunk; and in good preparations, nerve trunks in cross sections show numerous circular myelin sheaths, a structure lacking in all nerve end-organs so far observed.[17]

Observers: The observers were E. S. Rudisill and S. F. Cooper, with whom the work was carried through to completion; and S. Sanderson, D. E. Busby and C. R. Pendleton, with whom this part of the work was abandoned before completion.

Apparatus: The apparatus consisted of four parts: (1) An arm rest with a thin metal table above it, the height adjustable so that the metal would not lower the temperature of the skin; a square hole in the metal table allowed careful manipulation of a small exposed part of the forearm of the observer. (2) A marker.[18] (3) A double scale graduated along two perpendicular lines in ½-mm. steps, made of transparent celluloid, so that the coördinates of marks of cold or warm spots could be read with respect to two points previously tattooed in the skin. (4) The best histological apparatus obtainable.

Procedure. The procedure was to explore the skin (size of area 1 x 2 cm.), by continuous motion in one direction along parallel lines (¼–⅓ mm. apart), mark the spots, read the coördinates, enlarge ten times and transfer the coördinates of every observation to a single sheet of paper. Clusters of

[16] A. A. Böhm, M. von Davidoff, and G. C. Huber, *A Textbook of Histology*, 1926, 170.
[17] A. Ruffini, *op. cit.*, 438.
[18] C. R. Pendleton, Instrument for marking the temperature spots on the skin. *J. Exper. Psychol.*, 8, 1925, 471-474.

observations were assumed as the most probable location of the temperature organs. Pieces of skin over 1 x 2 cm., including both tattoo points, were then removed by the experimental dermatologist of the Medical School, care being taken to get all of the lower layers of the skin; then paraffin serial sections were made.[19] Some were stained with Mallory's aniline blue, others with Delafield's hematoxylin and Van Gieson's solution.

Results. No nerve end-organs were found. Certain bodies observed in preliminary studies of tissue taken from autopsies, thought to be end-bulbs, turned out to be cross sections of nerve trunks. All bodies in the two biopsies studied, resembling end-bulbs were nerve trunks. This was demonstrated both by the presence of myelin sheaths and by serial section study. Consequently no comparison of the two patterns, one of temperature spots, the other of end organs was possible.

One of two conditions is possible here—either there are no end-bulbs in the hairy parts of the skin, or the technique used was faulty. In regard to the first possibility, Von Frey[20] found one in the upper arm, but Ruffini says that there is no adequate knowledge of this region.[21] In regard to the second possibility, all we can say is that we used the best technique available. The results of this part of the work quite naturally lead to the search for specific end-organs in the hairy regions of the skin.

II. Search for Krause End-Bulbs and Ruffini Cylinders in the Hairy Regions of the Skin

In the second part of the work we used skin specimens from human legs taken immediately after amputations; specimens from the palms, the skin of the abdomen, and the conjunctiva from posted bodies; and specimens of palmar and toe pads and of the conjunctiva from the cat.

Procedure: We tried several fixing fluids—10% formalin, Bouin's, Fleming's, and Allen's. The latter was chosen as possibly better than the others, though Bouin's was used as an alternate.

Twelve blocks of human skin, taken from three different amputations, trimmed to 1 sq. cm., after fixation; a half-dozen blocks of smaller size from abdomen; three or four from fingers and palms, one from human conjunctiva, one from cat's conjunctiva, one cat's toe pad, and one cat's palmar pad, were used. The blocks of skin taken from amputations were imbedded as described above and sectioned, some normal, some tangential,

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[19]As paraffin sections of human skin are difficult to make, and as none of the published formulae worked well enough for serial sections, that finally adopted as the best is here offered. Fix for 1½-2 hrs. in Allen's fluid, B15; wash 24 hrs. in tap water; wash for 5-6 days in several changes of 50% alcohol; then 70% alcohol, 45 min.; 85% alcohol, 45 min.; 90% alcohol, 60 min.; 3 changes of absolute alcohol of 2, 3, and 3½ hrs. each; 3 changes of chloroform of 4, 8½, and 5 hrs. each; chloroform paraffin 3 hrs; German paraffin melting at 60°C. 3 hrs.; imbed in paraffin melting at about 55°C. With the razor in the best condition, a block of tissue, trimmed after fixation to 1 sq. cm., 4 mm. thick, may be cut without shifting the razor, and a serial section containing over a thousand sections mounted without losing or marring one.

[20]M. von Frey, *op. cit.*, 182.

[21]A. Ruffini, *op. cit.*, 425.

and mounted on slides. Samples of all blocks were stained, and two normal sections, seemingly the best were completely stained and finished. Several stains were tried out, and one of these blocks containing over 1,000 sections was stained in iron haematoxylin, the other in iron haematoxylin and eosin. The blocks of other tissues were treated in similar manner, except that not so many slides were made. The blocks from posted bodies were not considered reliable, but they and the other blocks showed what was wanted in a few sections.

It is believed that any square centimeter of human skin taken just below the knee will contain at least one cold spot. So that the sections of fresh human skin, trimmed to 1 sq. cm. after it had shrunken in the fixing fluid, ought to show at least one cold receptor. These two serial sections were studied in an effort to locate such an organ. The other tissues, palm of human hand, fingers, and conjunctiva, and cat's conjunctiva, toe, and palmar pads, were studied for the sake of comparison.

Results. The results of this section of the study may be summarized as follows.

(1) No Golgi-Màzzoni, Krause, or Ruffini end-organs were discovered in the hairy parts of the human skin. (2) In the human palms, where end-bulbs are known to exist in number,[22] such bulbs were found, and with very little difficulty. They are rounded organized groups of connective tissue and nuclei, different from any of the other well-known elements of the corium, the nerves having no myelin sheaths. (3) In the human conjunctiva, the cat's conjunctiva, the cat's toe and palmar pads, no definite identification of the end-bulbs in the papillae could be made. The places where they are to be found are easily discoverable; some that are probably end-organs may be pointed out. But to say that the tissue in a certain papilla is or is not an end-bulb, seems, on the basis of this study, to be impossible. The reason is obvious on examination of the slides. The dermic papillae where these bodies lie simulate the shape of the end-bulbs so closely that to discriminate the papillae with end-bulbs from those without is too uncertain to be dependable.

On the basis of these results, the probabilities seem to be against the existence of end-bulbs in the lower and middle layers of the corium, *i.e.* Krause, Golgi-Màzzoni, Dogiel, Ruffini, or whatever other name they may have. While the probabilities are about equal, for and against their existence in the dermic papillae just under the epidermis.

Any one of the following conditions may exist. (1) Bulbous corpuscles may exist in the lower layers of the corium, but are so small or so slightly organized that they are confused with other tissues. (2) Bulbous corpuscles may exist in the dermic papillae and function as the cold receptor, in which case they would be much the same as the Meissner corpuscles—though smaller and less organized—supposedly functioning as the tac-

[22] A. Ruffini, *op. cit.*

tile organ in the hairless parts. (3) There may be some unsuspected error of technique which makes these forms undiscernable. As a result of these considerations, the next part of the work was undertaken.

III. Bielchowsky's Method of Staining the Skin

There are four or five distinct methods of staining nerve tissue. Krause and Ruffini used gold chloride. Dogiel used methylin blue. It is somewhat more reliable than gold chloride, but many losses occur in sectioning. It works best in intravitam staining. Golgi, Cajal, and others used chrome silver and its modifications. All these methods are seemingly unsuited for quantitative work. They stain certain nerve elements and leave others untouched. What they show is brilliant, what they leave unstained may not even be suspected. They were tried out at the beginning of these studies and found too uncertain for the work in hand. But they could be applied qualitatively to the hairy parts of the skin as they have been applied to the hairless parts. Another method of staining has been developed by Bielchowsky, in which silver is used in combination with an alkali. This is claimed by many to be the best of all nerve stains. The application of this method to peripheral nerves, however, is not so good.

Much of the literature on this subject has been examined for a suitable method, with no decisive results. Modifications of Sand,[23], Agduhr,[24] and Boeke,[25] were tried on the conjunctiva, the hairless and hairy parts taken from posted bodies with the result that the connective tissue stained as brown as the nerves and no discrimination was possible. Another modification, that of Schultz, is reported by Von Frey to be the best yet devised. Before this could be tried out, a method was discovered for measuring the depths of the cold receptors, and the work turned in this direction, leaving many loose ends, some of which it is hoped will be taken up again. The greater part of the above is to be taken as a sketch rather than a set of scientific conclusions.

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[23]R. Sand, Une methode simple et elective de coloration des neurofibrilles et des cylindre-axes, *Zsch. wiss. Mikr.*, 28, 1911, 500.

[24]Erik Agduhr, Über Stückfärbung mit Bielchowskys Silberimprägnationsmethode. Einige Modifikationen, *Zsch. wiss. Mikr.*, 34, 1917, 1-99.

[25]J. Boeke, Die motorische Endplatte bei den höheren Vertebraten, ihre Entwickelung, Form und Zusammenhang mit der Muskelfaser, *Anat. Anz.*, 35, 1909, 196.

IV. Determination of the most probable Depth of the Cold Receptors

Strughold's fusion beads[26] suggested a method for measuring the depth of the cold organs, which was the next phase of the problem attacked.

Strughold used small beads of different sizes on the end of fine copper wire leads to show that the relative depths of the Krause end-bulbs below the conjunctiva corresponded to the size of the fusion beads, *i.e.* the amount of heat extracted. The variation in the size of his beads, however, seems out of proportion to the different thicknesses of tissue involved although he made allowances for several physical conditions. This irrelation would seem to be due to a factor which was not considered, namely, the size of the wire lead by which the bead is suspended. In this stimulus there are two masses of metal which extract heat, the copper bead and the copper wire lead. This latter was ignored, although it was possibly a greater factor than the bead itself in the smallest stimulus used, while in the largest it was probably only a small fraction of the total. In other words, there are two factors which make the stimulus in this series of instruments, one is constant and was incorrectly ignored and the other is variable and was used exclusively as a basis of calculation.

Method of Procedure. The method used in the present set of experiments takes advantage of the fact that copper cylinders of varying diameter and sufficient length will have only one variable, the square of the diameter. The length ceases to be a variable if it is sufficiently great, because the flow of heat is such that after a certain distance the copper wire or cylinder is at room temperature although one end be maintained constantly at a higher temperature. The physicists tell us that, with the slight difference in temperature involved and the size of the cylinders used, the length need be only several times the diameter.

If then a set of copper cylinders of varying diameter and sufficient length be used, and the room and the skin surface be kept at constant temperatures, the amount of heat extracted will vary according to the square of the diameter of the cylinders, and consequently the amount of heat extraction necessary to stimulate the cold organ can be measured. This amount will be composed of two quantities: (1) that required to arouse the organ itself in its uncovered state, which to begin with, may be assumed constant for each individual in a state of health and for a short period of time; and (2) that required to lower the temperature of the tissues lying between the surface of the skin and the organ itself. This latter quantity will vary according to the cube of the depth of the receptor, provided that the conditions, to be considered later, remain the same.

The first method for measuring the depth of the cold body is to determine the limen of a cold spot, remove a known quantity of epidermis and determine the limen again. From the three quantities determined, the depth of the cold body below the surface can be measured. The formula will be $x = c/(1-d)$ in which c is the thickness of the epidermis removed and d is the ratio of the two limens. The constants necessary to construct the equation are eliminated, but the constant representing that part of the heat extraction necessary to stimulate the uncovered cold receptor must be ignored in this method. If it is small the results will not be appreciably affected; in any case the value x will fall below the cold organ unless this quantity be zero.

A second method which offers at least a possibility of accounting for this last quantity is as follows. Determine the limen of a number of cold spots

[26] Hubert Strughold, *op. cit.*, 202.

in different parts of the body where the skin varies in thickness. Next measure the thickness of the several layers of the skin over the several spots. Then compare the ratios of limens to the ratios of thickness. If the several layers of the skin should vary according to the total thickness, nothing could be gained; but as this seems not to be the case[27] that set of ratios of thicknesses which is nearest to the ratios of the limens will be indicated. If then a constant determinable quantity be subtracted from the limens, to represent that necessary to stimulate the receptor itself, leaving a second set of quantities and ratios which compare more favorably with the ratios of depths of certain layers of skin, then the depths and layers of skin thus found are indicated for the location of the receptors. And the quantity extracted is a measure of the amount of heat extraction necessary to arouse the uncovered cold organ.

The second method, however, gives only the relative depth of the cold organ. This body might lie two or three times as deep as the position indicated. A comparison of this depth with that found by the first method will remove the uncertainty.

The cylinders were made of copper wire drawn and ground to the proper diameters and mounted by two fine copper wires on round brass key tags stamped with the diameter of the cylinders. They are in two series, one running from 4.00 mm.–0.20 mm. in steps of 0.05 mm.; the other running from 0.26 mm.–0.08 mm. in steps of 0.02 mm. The method of construction allows an accuracy of at least 0.005 mm. but the original wires were not strictly round and the irregularities could not easily be removed. The practice was to take the average of the largest and the smallest diameter as the nominal diameter.

The sizes turned out to be unexpectedly small. Strughold's smallest reported bead had a diameter of 0.22 mm. on a wire 0.10–0.15 mm. The smallest cylinder reported cold was 0.08 mm. which happened 7 times, while the cylinders having a diameter of 0.14 and lower were reported cold 37 times. The surface of the skin was moistened with a glycerin solution to correspond somewhat with the condition of the conjunctiva. Assuming that the cold organ in Strughold's experiment lay just below the conjunctiva, the cold organ in the observations just mentioned—on the back of the hand—must lie well up in the epidermis. The thickness of the conjunctiva varies from 45–80μ.[28] The thickness of the several layers of the epidermis over the spots which gave the above observations may be seen in Table IV. Biopsy No. I and VI where the stratum corneum is roughly 67μ and 63μ respectively.

Observers. The observers in this series were H. S. Oberly (5 limens and 3 biopsies), and C. R. Pendleton (26 limens and 3 biopsies).

Results. The magnitude of the limen is affected by a number of conditions. The state of attention is obvious, but the reported pleasing stroke of a camel's hair brush with the warm glycerin solution had a surprising though slight effect in lowering the limen. Fatigue is the hardest factor to deal with. There seem to be two kinds. The first is slight and heightens the limen, a slight rest will often restore the original state. It is important to note here that if the series be started too high above the limen, the organ will become fatigued before the true

[27] V. Drosdoff, De la mensuration de l'epiderme dans les differentes parties du humain et des rapports entre son épaisseur et la sensibilité electrocutanée, *Arch. de Phys. normal et pathol.*, 2 Ser. 6, 1879, 117-134. A. Koelliker, *Handbuch der Gewebelehre des Menschen*, 1889, 161, 203.

[28] G. A. Piersol, *op. cit.*, 1451.

limen is reached. Apparently subliminal stimuli will also fatigue the organ. Both were avoided by using short series, starting at or near the lowest perceived stimulus. The limen is then found to drop slowly until a fairly even position is attained. After a few series the limen will again jump upwards and the cold spot will not return to normal for some time. This is the second kind of fatigue mentioned above. Usually two or more spots were studied by turns, so that little time was lost while certain spots were resting. In calculating the

FIG. 1. SUCCESSIVE STAGES OF THE LIMEN OF SPOT NO. 5

limens, those series which seemed to be plainly affected by fatigue were discarded but no rule has been evolved to determine which to rule out. Fig. 1 illustrates how the limen fell as successive stages of fatigue were eliminated.

A number of physiological conditions seem to affect the limen. A chilly feeling, cold hands, slight drafts, a cold surface under the hand all seemed to have marked effect on the limen. A woolen cloth was placed under the hand and arm and as comfortable position as possible obtained. Even then a cramped feeling from a twisted arm in the palm-up position sometimes interfered. After tattooing the location of a spot, it was necessary to wait sometimes for a week for the injured

cuticle to recover completely; a short rest after walking seemed to be necessary, and the amount of caffeine used for breakfast seemed to affect it.

The temperature of the room was kept at 20° C ± 0.5 by opening and closing a window and waiting until a thermometer, graduated to tenths of a degree, standing as close as practicable to the cylinders and the observer, recorded a temperature within the required limits. The surface of the skin was moistened with a mixture of glycerin three parts and water two parts; the glycerin to prevent heat extraction from evaporation; the water to give greater fluidity; and the mixture to even out certain irregularities in the surface of the skin, and to approximate the condition of the conjunctiva, with which comparisons have already been made. The accurate placement of the stimulus is more serious than might be supposed. To place a cylinder of 1/10 or even 2/10 of a millimeter in diameter on a point is not easy. Supposing that it is often missed, a few trials for each cylinder were allowed, when there was no response. The time between applications was about a half second and the length of the application varied from an instant for the smallest to about one second for the largest. The latter was found necessary; the former seemed to be useful in avoiding fatigue.

Table I gives the limens determined in different parts of the arm and hand of two *O*s, calculated by a form of Müller's method of Least Differences.

It should be noted that, in those cases where no limen is given, but only the lowest perceived, the figures mean practically nothing. The limens probably were never reached owing to fatigue or to some condition of injury. The figures are given merely to complete the record.

In determining the depth of the cold organ by the first method, the cuticle is ground down with sand paper, after the limen is determined. Care must be taken here to prevent injury to the sensitive tissues since this heightens the limen. Accordingly the sensitivity was tested occasionally as the grinding went on. Table II indicates results from this process in two cases.

The next step is to excise two blocks of cuticle, one from the ground portion and one from the normal skin as near and as similar as possible to the first, and measure the thickness of the strata in the two cases, by slides prepared according to the formula given above. The last column in Table II gives these results. It will be seen that $c = 166\mu$, $d = 0.3305/0.6179$; from which $x = 357\mu$. That is, the depth of the cold organ is 357μ below the surface of the epidermis. By comparison with Table III, Biopsy V, column 7, which shows the thickness of the stratum corneum, it will be seen that this position is about 24μ, below the bottom of the stratum corneum or near the top of the living part of the skin.

A somewhat simpler variation for determining the value c is to slice off a piece of the epidermis after the exact location

of the spot has been marked, section and measure the thickness of the epidermis removed and proceed as above. This, however, has not been tried.

TABLE I

LIMENS OF COLD SPOTS IN DIFFERENT PARTS OF THE HAND AND ARM

O	Location	Spot no.	No. of series	Limen	Lowest perceived	Biopsy no.
	Center arm	1	2	0.2250	0.20	
		2	6	0.1666	0.14	
		3	0		0.20	
		4	0		0.70	
	Inner arm	1	4	0.1450	0.15	
		2	5	0.1550	0.12	
		3	6	0.1900	0.18	
		1*	1		0.22	
		2	17	0.0953	0.08	I
		3	7	0.1859	0.18	
		4	0		0.65	
		5†	0		1.30	
	Back of hand	6	8	0.1775	0.18	
Pendleton		7	0		0.18	
		8	6	0.1200	0.12	
		9	3	0.3266	0.22	
		10	0		0.20	
		11	3	0.1600	0.16	
		12	0		0.20	
	Palm of thumb	1	17	0.4350	0.40	II
		2	15	0.9433	0.65	
	Center of Palm	3‡	0		1.30	
		4‡	0		1.30	
		5§	0		1.75	
	Heel of palm	6	8	1.1750	0.65	
		7	16	1.1750	0.95	III
	Back of hand	1	3	0.1733	0.18	
		2	11	0.1036	0.08	IV
(	Palm of thumb	3	8	0.3875	0.30	
		4	2	0.5000	0.50	
	Heel of palm	5	21	0.4857	0.20	V

*Moved twice, *i.e.* three locations within 2 mm.
†Apparently injured in tattoo, it was insensitive for some time.
‡Apparently inflamed at the time.
§Not certain of location of spot.

In the second method for determining the depth of the cold organ the limens are determined and the thickness of the overlying tissues measured as described above. But here the cor-

Table II

Successive Limens in Grinding Process

O	Location	Spot No.	No. of grinding	No. of series	Limen	Lowest perceived	Limen raised to ⅔ power	Biopsy	Thickness of stratum corneum
Oberly	Heel of palm	5	Normal	21	0.4857	0.30	0.6197	V	333μ
			A			0.50			
			B			0.30			
			Final	2	0.1900	0.20	0.3305		167μ
Pendleton	Heel of palm	6	Normal	8	1.1750	0.65			
			A			0.35			
			B	4	0.4500	0.35			
			Final	4	0.4250	0.35			

responding layers of skin of different thickness are measured, the ratios found which are nearest to the ratios of the relative depths, *i.e.* the limens raised to the two-thirds power. Table III is a set of limens from 6 cold spots compared with the thickness of different layers of epidermis under them. Column 4 gives the limen in terms of the diameter; column 5, the diameter raised to the two-thirds power; and column 6, the same value as column 5 in ratios, *i.e.* every set of figures is divided by the value obtained in Biopsy I. Columns 7, 9, 11, and 13 give the measured thickness of different parts of the epidermis, while columns 8, 10, 12, and 14 give the ratios of the same values.

It will be noted that column 14, the bottom of the papillae—the juncture of epidermis and the corium—agrees best with the ratios of the limens, *i.e.* the sum of the deviations is smaller. But it is to be noted that the cold organ is somewhere above this point, the distance depending on the amount of the stimulus used in exciting the organ itself. If certain arbitrary amounts be subtracted, to account for this factor, other and higher points of 'best agreement' will be obtained. Table IV is a table of three such subtractions. It will here be seen that as the amount increases to about 0.1000 the sum of the deviations becomes least, further increase enlarges the sum. For this amount that depth of epidermis has the least sum of deviations which corresponds to the bottom of the stratum corneum. This agrees with the depth found by the first method, and with the above comparison with Strughold's data.

But if 0.1000 is the amount necessary to arouse the cold organ itself, this amount must be subtracted from the two members of the ratio calculation above. The quantity d then becomes 0.2305/0.5179, c remains the same, so that x (formula on p. 359 above) becomes 300μ below the surface of the skin. A comparison with the thickness of the strata given in Table IV, Biopsy V, column 7, places this point just above the bottom of the stratum corneum. As the number of observations is small, this calculation must be considered in connection with results from the first method. A position just below the bottom of the stratum corneum, in the top of the stratum malpighi, where sensitivity is generally believed to begin, seems most likely.

At this point it should be noted that the cold spots studied were those having the smallest limens, which are believed to be those in which the cold organs are more superficial. The other spots whose limens are higher may be supposed to lie lower down in the stratum malpighi. From Table III, Biopsy I, column 13, it may be seen that the actual depth of the bottom of the stratum malpighi (bottom of papillae) on the back of the

Table III

Limens Compared with Thickness of the Strata of the Epidermis

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Biopsy	Location of Cold Spot	No. of Series	First Limen			Stratum Corneum		Top of Papillae		Center of Papillae		Bottom of Papillae		Alternate Limen	
			Diam.	⅔ power	Ratio to I	μ	ratio	μ	ratio	μ	ratio	μ	ratio	⅔ power	Ratio to I
I	CRP 2 Back of hand	17	0.0953	0.2086	1.00	67	1.00	83	1.00	125	1.00	167	1.00	0.1086	1.00
II	CRP 1 Palm (thumb)	17	0.4350	0.5741	2.75	283	4.25	358	4.30	450	3.60	500	3.00	0.4741	4.37
III	CRP 7 Palm (heel)	16	0.1750	1.113	5.34	600	9.00	633	7.60	683	5.47	750	4.50	1.013	9.33
IV	SO 5 Palm ground	2	0.1900	0.3305	1.58	167	2.50	233	2.80	283	2.27	333	2.00	0.2305	2.12
V*	SO 5 Palm natural	21	0.4857	0.6179	2.96	333	5.00	392	4.70	450	3.60	516	3.10	0.5179	4.77
VI	SO 2 Back of hand	11	0.1036	0.2206	1.06	63	0.95	105	1.25	130	1.04	155	0.93	0.1206	1.11

*This biopsy was taken a short distance from spot 5, from tissue of exactly the same external character, which had not been ground. The limens in this line were taken from spot 5 before grinding.

hand of one *O* (Pendleton) is 167μ. This corresponds to a cylinder of 0.19 mm. diam. All the limens of this region except one fall within this limit. Laboratory notes show that this spot was dull and hard to measure.

TABLE IV

LIMENS COMPARED WITH THICKNESS OF EPIDERMIS AFTER SUBTRACTING QUANTITIES TO REPRESENT THAT AMOUNT NECESSARY TO AROUSE THE ORGAN ITSELF

Ratio	Biopsy	Limen ratio	Deviations			
			Stratum Corneum	Top of papillae	Center of papillae	Bottom of papillae
original	I	1.00	0.00	0.00	0.00	0.00
	II	2.75	1.50	1.55	0.85	0.25
	III	5.34	3.66	1.26	0.13	0.84
	IV	1.58	0.92	0.22	0.69	0.42
	V	2.96	2.04	1.74	0.64	0.14
	VI	1.06	0.11	0.19	0.02	0.13
	Sum of deviations		8.23	4.96	2.33	1.78
0.05 subtracted	I	1.00	0.00	0.00	0.00	0.00
	II	3.30	0.95	1.00	0.30	0.30
	III	6.70	2.30	0.90	1.23	2.20
	IV	1.77	0.73	1.03	0.50	0.23
	V	3.58	1.42	1.12	0.02	0.48
	VI	1.08	0.13	0.17	0.04	0.15
	Sum of deviations		5.53	4.22	2.09	3.36
0.10 subtracted	I	1.00	0.00	0.00	0.00	0.00
	II	4.37	0.12	0.07	0.77	1.37
	III	9.33	0.33	1.73	3.86	4.83
	IV	2.12	0.38	0.68	0.15	0.12
	V	4.77	0.23	0.07	1.17	1.67
	VI	1.11	0.16	0.14	0.07	0.18
	Sum of deviations		1.22	2.69	6.02	8.17
0.15 subtracted	I	1.00	0.00	0.00	0.00	0.00
	II	7.24	2.99	2.94	3.64	4.24
	III	16.43	7.43	8.83	10.96	11.93
	IV	3.08	0.58	0.28	0.81	1.08
	V	7.98	2.98	3.28	4.38	4.88
	VI	1.20	0.25	0.05	0.16	0.27
	Sum of deviations		14.23	15.38	19.95	22.40

Of the factors which would tend to vitiate the results the most obvious are the crude methods of calculating the limens and the great variability in measuring the depths. In calculat-

ing the limens, fatigue prevents anything like a determination of the limen by the $\Phi(\gamma)$ hypothesis. The method of least differences is the best method that could be used. With plenty of time much more accurate results could be obtained, but these probably would not alter the stratum in which the cold organ is found to lie. The measurements on the depths of the layers of the skin are averages of four positions taken, as near as possible, to the actual position of the cold spot.

Other conditions which might alter the calculated depth are: (1) a change in the direction of the flow of heat, *i.e.* it might flow more tangentially in some strata than in others; (2) a difference in the specific heat of different strata; and (3) nearness to blood vessels. All these must be disregarded at present. But the fact that the calculations place the cold organ so near the surface of the skin, and well above those strata which differ markedly in their physical and histological structure, makes these conditions seem less serious.

It seems unlikely that a cold spot near a set of capillaries could be lowered appreciably by a stimulus as small as has been found sufficient to arouse the cold body. Cylinder 0.08 mm. has been reported cold 7 times, and 0.10 mm. and lower 24 times. The papillae under these spots measure roughly 0.05 mm. in diam. They have a net work of capillaries in them. If a part of this stimulus goes to lower the temperature of the intervening tissues the remainder would seem wholly inadequate to lower the temperature of the cold body near the blood vessels. This is even more unlikely if the cold organ be of the bulb type which has blood vessels in it and around it;[29] or if the cold body lies below a plexus of blood vessels.

V. Histological Examination of the Skin

Stimulation of the cold spots after the biopsy operation had healed showed a return of the cold spot, after some time (roughly 60 days), in every case at the edge of the incision although the original spot was in the center, as accurately as the eye could see. The incisions were made by means of a two millimeter dermic punch. In every case a scar was left, in some cases an almost perfect circle.

In all but two of the biopsies, the spots for some time after healing, *i.e.* 15 to 30 days, were insensitive to cold at 0°C. One of the two exceptions, Biopsy II, was sensitive at this temperature before the blood clot had disappeared. There are now two spots along the edge of the scar, about one millimeter apart, from which it seems that there were two cold spots near together here,

[29] A. Ruffini, *op. cit.*

one remaining just beyond the edge of the incision, and the other removed, because the original cold spot lay between two tattoo spots, both of which appear in the sections. Of possible interest here is that this spot was unusually painful in the excision and that microscopic examination showed a large nerve trunk under it. The other of the two spots seemed to respond at 0°C as soon as healing was completed. This response was vague and uncertain, however, and opportunity to make further careful examination was lacking. Sixty days later (roughly) this spot was clearly located on the edge of the scar. From this it may be supposed that the doubtful cold sensation reported was an error. It is unfortunate that this cannot be stated more emphatically. The biopsy removed the epidermis with only a very small layer of the corium (about 17μ). The significance of this fact was not appreciated until after the return of the cold spot on the edge of the scar. When the spots return the limens are plainly higher but exact measurements have not been made. They seem to lower as time goes on but this again has not been measured.

In examining the slides made from these biopsies, especial efforts have been made to find end-bulbs. Not one has been discovered, except Meissner's corpuscles in Biopsies IV and V. Limitation on the significance of this should be placed in accordance with what has been said in Sections I and II. The localization of the cold spots is, however, the most accurate known to the writer. The small size of the stimulating instrument on the back of the hand makes accurate placement necessary. The elimination of unnecessarily strong stimuli makes stimulation from a distance unlikely while the elimination of fatigue factors tends to prevent them failing to appear where they are.[30]

Biopsy I is a good example; this was accurately localized after the tattoo point had been made; than a second tattoo point was inserted. The cold spot was found to be between these two spots and a little to one side. A preliminary shallow cut was made with the punch and a drawing of the circle and three points near the center made. The excision was then made and after fixation the two tattoo points could be seen and part of the preliminary circle, the final cut having been made slightly to one side of the preliminary marker. The two tattoo points and the cold spot remaining in the form of a roughly equilateral triangle one half millimeter on a side in the center of the 2 mm.

[30]It may be suggested that possibly some of the unaccountable absence of marked cold spots on certain occasions is due to their having been fatigued by cold from nearby applications. Only two of the 36 spots studied by this method have ever failed to appear, so far as laboratory notes go. One responded only once. It was plainly inflamed from the tattoo mark. The other seemed to be dull. Both were dropped from further study.

circle. This biopsy was insensitive to cold for some time after complete healing and was deep enough to take out all or nearly all of the corium.

There is reason to believe that the surface spot was removed in every case but the depth varied from just below the epidermis in Biopsy III to probably the full depth of the corium in Biopsies I and IV. It is quite possible that Golgi-Màzzoni corpuscles may have lain at the bottom of the corium where Ruffini says they are found, and were not removed.

Conclusions

The evidence of these studies is against the Golgi-Màzzoni end-bulbs being the cold receptors. They have not been found in the hairy parts of the skin nor in the biopsies of the hairless parts, Biopsy II having been quite deep. If they are found mostly at the juncture of the corium and the tela subcutania, as Ruffini contends, they would be far too deep and covered with too many capillaries to receive the minimal cold stimuli. If they are found in the papillae as Dogiel reports they come under the same category as the Krause end-bulbs mentioned below.

No evidence was found as to the existence or non-existence of the Krause end bulbs in the dermic papillae. If they or Golgi-Màzzoni end-bulbs are in the papillae, there is quite a question whether they are not the same as the Meissner corpuscles. In any case they are deeper than the calculated positions of the cold bodies.

There is no evidence here to support Haggqvist's findings of smooth muscle fibers under the cold spots.

All the evidence points to the supposition that the cold receptors lie in stratum malpighi; some near the top and some deeper down; and that the difference in the limens is due mainly to the thickness of the overlying tissues. In the stratum malpighi there are two forms of nerve endings, the well-known free nerve endings discovered by Langerhans in 1868, and the so-called hederiform nerve endings of Ranvier. As the latter are not so well known, and as there is some reason to suppose that they may be the cold organ, a brief description of them, taken from Ruffini[31] follows.

The hederiform nerve endings are basket-like in character with the concavity turned towards the outer surface of the skin; the basket usually contains one of the prickle cells of the Malpighi layer somewhat modified in its histological characters. These cells were discovered by Merkel in 1875, called *Tastzellen* and thought to be peripheral nerve cells. But Ranvier in 1880, on the basis of more careful studies, concluded that they were epithelial cells having a special relation to these nerve endings. He called them hederiform terminations from their supposed resemblance to ivy.

[31] Ruffini, *op. cit.*

But Dogiel with better methods found this description not wholly correct. Rather they are basket-like, composed of slender, varicose, non-medullated fibers interlacing to form a net work around the base of one of the prickle cells. These fibers arise from medullated fibers of the superficial plexus of the skin. Accepting this description as the most probable, Ruffini proposes that they be called the intra-epithelial baskets of Dogiel. They lie near the ducts of the sweat glands and are considered the most recent form of nerve endings in the vertebrate epidermis, being found only in mammals.

It is not unreasonable to suppose that the sense of cold with its cold organ appears for the first time with the warm-blooded animals. Since the free nerve endings of Langerhans seem to be the oldest form of nerve endings in the vertebrate epidermis and the hederiform endings are considered the latest, it seems not improbable that the latter are the cold organs. The relation of these nerve end-organs to the sweat gland ducts offers an opportunity to test this supposition.